Mag-Bind® Stool DNA Kit

M4015-00	5 preps
M4015-01	50 preps
M4015-02	200 preps

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Mag-Bind® Stool DNA Kit

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OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The Mag-Bind® Stool DNA Kit allows rapid and reliable isolation of high-quality total DNA from fresh and frozen stool samples. Up to 200 mg stool samples can be processed in less than 60 minutes. The system combines the reversible nucleic acid binding properties of Mag-Bind® particles with the efficiency of HTR Reagent to eliminate humic acid, polysaccharides, phenolic compounds, and enzyme inhibitors from stool samples. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions, thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Overview

Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. The Mag-Bind® Stool DNA Kit uses a unique HTR Reagent and SP2 Buffer that can remove inhibitory substances from stool samples.

If using the Mag-Bind® Stool DNA Kit for the first time, please read this booklet to become familiar with the procedures. Frozen or fresh stool samples are homogenized and then lysed in a specially formulated buffer containing detergent. Proteins, polysaccharides, and cellular debris are subsequently precipitated with SP2 Buffer after a heat-freeze step. Contaminants are further removed using the HTR Reagent by a quick centrifuge step. Binding conditions are then adjusted and the DNA is selectively bind to the surface of Mag-Bind® Particles. Three rapid wash steps remove trace contaminants, and pure DNA is eluted in DNA Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

- Proteinase K is now supplied in a liquid form eliminating the resuspension step prior to use. Proteinase K Solution can be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

Kit Contents

Product	M4015-00	M4015-01	M4015-02
Preparations	5 preps	50 preps	200 preps
Mag-Bind® Particles CND	250 µL	1.2 mL	5.5 mL
SLX-Mlus Buffer	5 mL	50 mL	200 mL
DS Buffer	0.5 mL	5 mL	20 mL
SP2 Buffer	1.2 mL	12 mL	50 mL
HTR Reagent	1.2 mL	12 mL	50 mL
XP2 Buffer	6 mL	60 mL	250 mL
SPM Wash Buffer	3 mL	30 mL	60 mL
Binding Enhancer	60 µL	0.6 mL	2.4 mL
Proteinase K Solution	120 µL	1.2 mL	4.4 mL
Glass Beads	1.2 g	12 g	45 g
Elution Buffer	2 mL	15 mL	60 mL
User Manual	✓	✓	✓

Storage and Stability

All of the Mag-Bind® Stool DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles CND, HTR Reagent, and Binding Enhancer should be stored at 2-8°C for long-term use. Proteinase K can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K at 2-8°C. During shipment or storage in cool ambient conditions, precipitates may form in DS Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M4015-00	7 mL
M4015-01	70 mL
M4015-02	140 mL

Mag-Bind® Stool DNA Kit Protocol - Human DNA Detection

Materials and Equipment to be Supplied by User:

- Magnetic Separation Device for 1.5 mL Tubes (Cat# MSD-02)
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water bath capable of 65°C
- 100% Ethanol
- OPTIONAL: RNase A stock solution at 20 mg/mL
- Ice Bucket

Before Starting:

- Heat the water bath to 65°C
- Prepare the SPM Wash Buffer according to the instructions in the Preparing Reagents section on Page 4
- Prepare an ice bucket

1. Weigh up to 200 mg stool sample in a 1.5 mL or 2 mL microcentrifuge tube and place the tube on ice.

2. Add 800 µL SLX-Mlus Buffer. Vortex at maximum speed for 5 minutes or until the stool sample is thoroughly homogenized.

   Note: If the sample is liquid, pipet 200 µL sample into the microcentrifuge tube. Cut the end of the pipet tip to make pipetting easier. If the sample is frozen, use a spatula to scrape the sample into the tube. Do not thaw the frozen sample until the SLX-Mlus Buffer is added into the tube.

3. Add 80 µL DS Buffer. Vortex for 10 seconds to mix thoroughly.

4. Centrifuge at maximum speed (≥13,000 x *g*) for 5 minutes to pellet the stool particles.

5. Transfer 600 µL supernatant into a new 1.5 mL tube.

   Note: Cut the end of the pipet tips to make pipetting easier for viscous stool samples.

6. Add 20 µL Proteinase K Solution. Incubate at 65°C for 10 minutes.

7. Add 200 µL SP2 Buffer and 200 µL HTR Reagent . Vortex for 30 seconds to mix thoroughly.

   Important: HTR Reagent must be thoroughly resuspended before being dispense from bottle.

   Tip: Cut the end of the pipet tips to make pipetting the HTR Reagent easier.

8. Let sit on ice for 5 minutes.

9. Centrifuge at maximum speed (≥13,000 x *g*) for 5 minutes at room temperature.

10. Transfer 600 µL cleared supernatant to a new 1.5 mL tube.

Optional: If RNA-free DNA is required, add 10 µL RNase A (not supplied). Incubate at 65°C for 3 minutes.

11. Add 600 µL XP2 Buffer. Vortex for 10 seconds to mix thoroughly.

12. Add 20 µL Mag-Bind® Particles CND. Mix by pipetting 10-20 time or vortexing.

13. Let sit at room temperature for 5 minutes. Mix the sample several times during incubation by vortexing or pipetting up and down 10 times.

14. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

15. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

16. Remove the tube containing the Mag-Bind® Particles CND from the magnetic separation device.

17. Add 400 µL XP2 Buffer.

18. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times. Let sit at room temperature for 2 minutes. Mix the sample several times during incubation by vortexing or pipetting up and down 10 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CND.

19. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

20. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

21. Remove the tube containing the Mag-Bind® Particles CND from the magnetic separation device.

22. Add 400 µL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

23. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times. Let sit at room temperature for 1 minute. Mix the sample several times during incubation by vortexing or pipetting up and down 10 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CND.

24. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

25. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

26. Repeat Steps 21-25 for a second SPM Wash Buffer wash step.

27. Leave the tube on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CND. Remove any residual liquid with a pipettor.

28. Remove the tube containing the Mag-Bind® Particles CND from the magnetic separation device.

29. Add 50-200 µL Elution Buffer or nuclease-free water.

30. Resuspend the Mag-Bind® Particles CND by pipetting up and down 50 times or vortexing for 3 minutes.

31. Let sit at room temperature for 5-10 minutes.

    Note: Incubation at 65°C rather than room temperature will give a modest increase in DNA yield per elution.

32. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA to a clean 1.5 mL tube. Store the DNA at -20°C.

    Note: A second elution may be performed using the first eluate.

Mag-Bind® Stool DNA Kit Protocol - Pathogen Detection

Materials and Equipment to be Supplied by User:

- Magnetic Separation Device for 1.5 mL Tubes (Cat# MSD-02)
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water bath capable of 65°C
- 100% Ethanol
- OPTIONAL: RNase A stock solution at 20 mg/mL
- Ice bucket

Before Starting:

- Heat the water bath to 65°C
- Prepare the SPM Wash Buffer according to the instructions in the Preparing Reagents section on Page 4
- Prepare an ice bucket

1. Weigh 50-100 mg stool sample in a 1.5 mL or 2 mL microcentrifuge tube containing 200 mg of glass beads and place the tube on ice.

   Note: If the sample is liquid, pipet 200 µL sample into the microcentrifuge tube. Cut the end of the pipet tip to make pipetting easier. If the sample is frozen, use a spatula to scrape the sample into the tube. Do not thaw the frozen sample until the SLX-Mlus Buffer is added into the tube.

2. Add 270 µL SLX-Mlus Buffer. Vortex at maximum speed for 5 minutes or until the stool sample is thoroughly homogenized.

3. Add 30 µL DS Buffer and 20 µL Proteinase K Solution. Vortex for 10 seconds to mix thoroughly.

4. Incubate at 65°C for 10 minutes (13 minutes if frozen). Mix sample twice during incubation by vortexing the tube.

Optional: For isolation of DNA from gram-positive bacteria, do a second incubation at 95°C for 5 minutes.

5. Let sit on ice for 2 minutes.

6. Add 100 µL SP2 Buffer and 100 µL HTR Reagent. Vortex for 30 seconds to mix thoroughly.

   Important: HTR Reagent must be thoroughly resuspended before being dispense from bottle.

   Tip: Cut the end of the pipet tips to make pipetting the HTR Reagent easier.

7. Let sit on ice for 5 minutes.

8. Centrifuge at maximum speed (≥13,000 x *g*) for 5 minutes at room temperature.

9. Transfer 300 µL cleared supernatant to a new 1.5 mL tube. Make sure not to disturb the pellet or transfer any debris.

Optional: If RNA-free DNA is required, add 10 µL RNase A (not supplied). Incubate at 65°C for 3 minutes.

10. Add 300 µL XP2 Buffer. Vortex for 10 seconds to mix thoroughly.

11. Add 20 µL Mag-Bind® Particles CND and 10 µL Binding Enhancer. Mix by pipetting up and down 10-20 times or vortexing for 30 seconds.

12. Let sit at room temperature for 5 minutes. Mix the sample several times during incubation by vortexing or pipetting up and down 10 times.

13. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

14. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

15. Remove the tube containing the Mag-Bind® Particles CND from the magnetic separation device.

16. Add 400 µL XP2 Buffer.

17. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times. Let sit at room temperature for 2 minutes. Mix the sample several times during incubation by vortexing or pipetting up and down 10 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CND.

18. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

19. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

20. Remove the tube containing the Mag-Bind® Particles CND from the magnetic separation device.

21. Add 400 µL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

22. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times. Let sit at room temperature for 1 minute. Mix the sample several times during incubation by vortexing or pipetting up and down 10 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CND.

23. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

24. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

25. Repeat Steps 20-24 for a second SPM wash step.

26. Leave the tube on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CND. Remove any residual liquid with a pipettor.

27. Remove the tube containing the Mag-Bind® Particles CND from the magnetic separation device.

28. Add 50-200 µL Elution Buffer or nuclease-free water.

29. Resuspend the Mag-Bind® Particles CND by pipetting up and down 50 times or vortexing for 3 minutes.

30. Let sit at room temperature for 5-10 minutes.

    Note: Incubation at 65°C rather than at room temperature will give a modest increase in DNA yield per elution.

31. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

32. Transfer the cleared supernatant containing purified DNA to a clean 1.5 mL tube. Store the DNA at -20°C.

    Note: A second elution may be performed using the first eluate.

Mag-Bind® Stool DNA Kit Protocol - Centrifugal Protocol

Note: Please read through previous sections of this manual before using this protocol.

1. Prepare samples by following the standard protocol in previous sections.

2. For all binding, washing, and elution steps, centrifuge at maximum speed (≥13,000 x *g*) for 1 minute to collect the Mag-Bind® Particles CND instead of using the magnetic separation device.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at 1-800-832-8896.

Problem	Cause	Solution
Low DNA yield	Incomplete disruption of starting material	Repeat the experiment with new sample, make sure the sample are completely disrupted and lysed.
	Sample stored incorrectly	Store the sample at -20°C.
	Loss of the Mag-Bind® Particles during operation	Carefully avoid removing any Mag-Bind® Particles during aspiration.
	DNA remains bound to Mag-Bind® Particles	Increase elution volume and incubate the sample at 65°C for 10 minutes.
	DNA washed off	Dilute SPM Wash Buffer by adding appropriate volume of ethanol prior to use (Page 4).
Problem	**Cause**	**Solution**
Problems in downstream applications	Ethanol carry-over	Dry the Mag-Bind® Particles before elution.
	BSA not added to PCR mixture	Add BSA to a final concentration of 0.1 µg/mL to the PCR mixture.
Problem	**Cause**	**Solution**
Low A260/280 ratio	Inefficient elimination of inhibitory compounds	Repeat with a new sample. Be sure to mix HTR Reagent thoroughly before use.

Ordering Information

The following components are available for purchase separately.
Call Toll Free at 1-800-832-8896

Buffer (Size)	Part Number
SPM Wash Buffer, 40 mL	PS014
Elution Buffer (EB Buffer), 100 mL	PDR048
Elution Buffer (EB Buffer), 500 mL	PD089
RNase A, 400 µL	AC117
RNase A, 5 mL	AC118
1.5 mL DNase/RNase-free Microcentrifuge Tubes	SSI-1210-00
2 mL DNase/RNase-free Microcentrifuge Tubes	SSI-1310-00
Magnetic Separation Device for 1.5 mL Tubes	MSD-02

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Notes: